JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1993, p. 2049–2056
0095-1137/93/082049-08$02.00/0

Vol. 31, No. 8

# Large-Scale Use of Polymerase Chain Reaction for Detection of *Mycobacterium tuberculosis* in a Routine Mycobacteriology Laboratory

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Received 2 March 1993/Accepted 3 May 1993

**We investigated the use of DNA amplification by the polymerase chain reaction (PCR) for detection of *Mycobacterium tuberculosis* from clinical specimens. Two-thirds of each sample was processed for smear and culture by standard methods, and one-third was submitted for DNA extraction, amplification of a 317-bp segment within the insertion element IS*6110*, and detection by agarose gel electrophoresis, hybridization, or both. DNA was prepared from over 5,000 samples, with 623 samples being culture positive for acid-fast bacilli. Of 218 specimens that were identified as *M. tuberculosis*, 181 (85%) were positive by PCR. In the *M. tuberculosis* culture-positive group, PCR was positive for 136 of 145 (94%) and 45 of 73 (62%) of the fluorochrome smear-positive and -negative specimens, respectively. Of 948 specimens that were either culture positive for mycobacteria other than *M. tuberculosis* or culture negative, 937 specimens were negative by PCR and 11 (1%) specimens initially appeared to be false positive for *M. tuberculosis*. The reasons for discrepant results varied; some errors were traced to the presence of an inhibitor in the specimen (7.3% in unselected specimens), nucleic acid contamination, low numbers of organisms in the specimen, antituberculosis therapy, and possible low-level nonspecific hybridization. In comparison with culture, the sensitivity, specificity, and positive predictive value were 83.5, 99.0, and 94.2%, respectively, for PCR. When PCR was corrected for DNA contamination, the presence of inhibitor, and culture-negative disease, the values became 86.1, 99.7, and 98.4%, respectively. If the results for multiple specimens submitted from the same patient are considered, no patient who had three or more sputum specimens tested would have been misdiagnosed.**

With the advent of multiply resistant *Mycobacterium tuberculosis* and the increased incidence of tuberculosis, the rapid diagnosis of tuberculosis has increasing public health significance (1, 23). In developed countries, increases are related not only to AIDS and homelessness but also to the aging of the population (11). Because of the ease of spread and the difficulty in treating this organism, it is prudent to isolate patients with acid-fast bacillus (AFB) disease until such time as the organism is shown not to be *M. tuberculosis*. Since isolation of patients is costly, it is advantageous to isolate only those with disease caused by *M. tuberculosis*. The most rapid method of identifying *M. tuberculosis* to the species level in clinical specimens is the polymerase chain reaction (PCR). It is known that the use of PCR for detecting mycobacteria from culture can be extremely sensitive and specific. The detection limits of *M. tuberculosis* by PCR from culture have been reported to be from as low as 1 to as high as 1,000 organisms (2, 15, 16, 20, 21, 28, 30, 31, 45). We have previously determined the detection limits of *M. tuberculosis* from culture to be as few as five organisms (37). In addition, amplification of the insertion segment IS*6110* that we used in the present study has been shown to be highly specific for *M. tuberculosis* (3, 13, 14). However, the usefulness of PCR in the diagnosis of tuberculosis by using a variety of unselected clinical specimens is not as clear. Studies with clinical specimens have differed not only in the PCR techniques, including the lysing method, the target nucleic acid, and the methods used to detect amplified products, but also in the number and type of samples used, making the reported sensitivities and specificities difficult to compare (2, 16, 24, 26, 31, 42). In addition, most of the clinical specimens were from patients in whom tuberculosis was already diagnosed (4, 9, 14, 26, 31, 35, 36). There is a need, then, to examine the technique on a large number of routine specimens as they are seen in a clinical laboratory. Since many of the lysing, detection, and extraction techniques are difficult and time-consuming, we chose to use in the present study a relatively simple system which used heat and detergent lysis, no DNA extraction, and detection of the product by agarose gel electrophoresis for all specimens and by nonradioactive hybridization for selected specimens. For part of the study, up to 100 unselected specimens which were routinely submitted to the mycobacteriology laboratory were examined each week. The patients from whom specimens showing discrepant results were obtained were evaluated for common factors which might cause the discrepancy, such as drug therapy or human immunodeficiency virus (HIV) status.

## MATERIALS AND METHODS

**Clinical specimens.** Clinical specimens were obtained from patients at the Veterans Affairs Medical Center in whom tuberculosis was suspected. The type (approximate percentage) of specimens submitted were as follows: sputum (50%),

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bronchial washing (8%), pleural fluid (4%), cerebrospinal fluid (CSF) (18%), urine (5%), tissue, skin, abscess and exudate (8%), blood (4%), bone marrow (2%), peritoneal fluid (1%), and feces (1%). All specimens were analyzed for growth of mycobacteria by using the BACTEC 460 system according to the manufacturer's instructions. Over 7,500 clinical specimens were processed for *Mycobacterium* culture and were subjected as appropriate to lysis to prepare the DNA for amplification. Because blood specimens were received in BACTEC 13A bottles, neither original smears nor PCR was performed on these specimens. Usually too little CSF was submitted to allow proper culture, smears, or PCR to be performed; in order not to compromise patient care, PCR was performed on only three of these specimens. A few other specimens, particularly tissue, were generally not amenable to division. Otherwise, all specimens during the study time were divided, with two-thirds used for culture and one-third used for the preparation of DNA. Specimens from contaminated sites were divided after decontamination and digestion with 2% NaOH (final concentration) in 0.5% *N*-acetyl-L-cysteine and concentration by centrifugation at 4,300 × *g* for 15 min in a refrigerated centrifuge. Following digestion, decontamination, and concentration (as appropriate), auramine-rhodamine acid-fast smears were made on all specimens except urine, CSF, and blood, and the smears were examined at ×500 power with a Nikon fluorescent microscope. Culture for mycobacteria was performed by inoculation of the digested material onto Lowenstein-Jensen and Middlebrook agar slants (one each) and into one BACTEC 12B bottle. The quantity of growth observed was tabulated as follows: <1+ = growth in BACTEC bottle only or <50 colonies on either slants, 1+ = 50 to 100 colonies, 2+ = 100 to 200 colonies, 3+ = 200 to 500 colonies, and 4+ = >500 colonies. Identification of all acid-fast isolates was performed by standard procedures (32). Over 5,000 clinical samples submitted for mycobacterial culture were lysed, and of these, 1,166 were amplified and analyzed by agarose gel electrophoresis, hybridization, or both. Selected agarose gel-positive and agarose gel-negative specimens and all agarose gel-negative specimens with discordant findings were analyzed by probe hybridization. This included all AFB smear- or culture-positive specimens. During one phase, all sequential specimens were examined.

**Preparation of DNA.** Clinical specimens obtained directly or after digestion were treated with heat and detergent as described previously (37, 40) to release the DNA. Approximately 1 ml of each sample was divided into aliquots placed into 1.5-ml Eppendorf tubes, and centrifuged at 12,600 × *g* for 5 min. The supernatant was discarded, and the pellet was washed once with a 1% Triton X-100 solution containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA and was then incubated in 150 μl of the same solution for 30 min at 100°C. After incubation, the sample tubes were centrifuged for 3 min and 125 μl of the supernatant was transferred into another labelled tube and stored at −70°C until further use.

**Amplification of mycobacterial DNA by PCR.** Oligonucleotides of 20 and 21 bp homologous for sequences in the insertion sequence IS*6110* specific for *M. tuberculosis* were used as primers for the PCR to amplify a 317-bp segment. The amplification procedure was performed as described previously (37). Briefly, 25 μl of the lysed specimen was added to 85 μl of the master reaction mixture containing 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 50 mM KCl, 200 μM (each) deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 0.5 μM (each) oligonucleotide primers, and 2.5 U of *Taq* polymerase (Promega Corp., Madison, Wis.). The mixture was subjected to 35 cycles of amplification with denaturation at 94°C (2.2 min) and annealing and extension at 68°C (3.25 min).

**Detection of amplified DNA.** For all specimens, an aliquot (25 μl) of the amplification product was examined by agarose gel electrophoresis to look for the expected 317-bp band. For selected specimens (all those with discrepant results and 400 others), the more sensitive method of hybridization with a labelled probe was used. For this, a 20-bp oligonucleotide probe homologous to the middle portion of the 317-bp sequence was labelled with alkaline phosphatase by a linker molecule according to the instructions of the manufacturer (Cambridge Research Biochemicals, Wilmington, Del.). Dot blot and Southern blots prepared by a standard procedure (33) were hybridized by using the probe described above as described previously (37).

**Evaluation of inhibition of PCR.** All available specimens that were positive for growth of *M. tuberculosis* but negative by PCR and a group of randomly selected AFB culture-positive and -negative specimens were analyzed for inhibition of PCR. Briefly, this was achieved by performing a second PCR on these lysates under the conditions described above, but with the addition of known template and primers. Detection of the product was performed by agarose gel electrophoresis as described above.

**Data analysis.** The Epi-Info 5.00 epidemiology software package was used for analysis of data.

## RESULTS

**Species distribution and smear results for cultures growing AFB.** Specimens that were submitted daily to the clinical laboratory were cultured for growth of mycobacteria and, if appropriate, were lysed to prepare DNA. Blood specimens which were received in BACTEC 12B bottles and specimens for which the quantity was insufficient (e.g., most CSF and tissue specimens) were cultured but not lysed. Of over 5,000 specimens that were cultured and lysed, 623 were positive for AFB (overall positive rate, 12%), of which 218 showed growth of *M. tuberculosis* (35%). Of these samples, 181 (85%) were positive by PCR. If the unusually high rate of *M. gordonae* isolation, which was due to contamination by BACTEC additives but which was later corrected for by filtration, is discounted, the percent positive cultures dropped to 10% and the percentage of *M. tuberculosis* increased to 42%.

The variation in culture and smear results for the identified species of mycobacteria is shown in Table 1. Two-thirds (66%) of the specimens which grew *M. tuberculosis* were positive by AFB staining. Of the specimens which were culture positive for mycobacteria other than *M. tuberculosis* (MOTT), 51% of the *M. kansasii* but only 14% of the *M. avium-M. intracellulare* or group III isolates not identified to species level, 6% of the rapid growers, and none of the other species were smear positive. When only sputum specimens are considered, 71% of the *M. tuberculosis* culture-positive specimens were smear positive, and of the smear-positive sputum specimens, 72% were *M. tuberculosis* (note that these percentages are nearly the same by coincidence, because there were 179 culture-positive sputum specimens and 176 smear-positive sputum specimens). There were two culture-negative, smear-positive, PCR-negative results for patients on antituberculosis medication (data not shown).

**Comparison of smear, culture, and PCR results.** Table 2 shows the overall PCR, culture, and smear results. PCR results were accurate for 181 specimens which grew *M.*

TABLE 1. Species distribution and smear results for cultures growing AFB

| Species | Specimen source | | | |
|---|---|---|---|---|
| | All | | Sputum | |
| | No. of specimens | No. (%) smear positive | No. of specimens | No. (%) smear positive |
| *M. tuberculosis* | 218 | 145 (66) | 179 | 127 (71) |
| *M. kansasii* | 53 | 27 (51) | 44 | 23 (52) |
| *M. avium-M. intracellulare*; group III | 194 | 27 (14) | 142 | 24 (17) |
| *M. fortuitum*; group IV | 35 | 2 (6) | 33 | 2 (6) |
| *M. xenopi* | 2 | 0 | 2 | 0 |
| *M. gordonae*; group II | 107 | 0 | 93 | 0 |
| Mixed, other | 14 | 0 | 7 | 0 |
| All | 623 | 200 | 500 | 176 (72[a]) |

[a] Percentage of which are *M. tuberculosis*.

*tuberculosis* and for 937 specimens which were culture negative for *M. tuberculosis*. Thirty-seven specimens representing 29 patients were negative by PCR but showed growth of *M. tuberculosis*, while 11 specimens from 10 patients were positive by PCR but were culture negative for *M. tuberculosis*. The smear positivity rates for all specimens were 75 and 24% for the PCR-positive and PCR-negative groups, respectively. In Table 3, the data for specimens culture positive for *M. tuberculosis* are presented in more detail. Of the 145 samples that were positive for *M. tuberculosis* by culture and smear, 136 (94%) were positive by PCR. Of the 73 samples that were *M. tuberculosis* culture positive and smear negative, 45 (62%) were positive by PCR. For sputum, of the 127 smear-positive specimens, 121 (95%) were PCR positive. Of the six sputum specimens that were missed by PCR, three were found to contain inhibitors, one had no evidence of inhibition, and two were not available for testing. All six of these specimens were from patients who had other specimens positive by PCR (see Table 7). Of the 52 smear-negative but *M. tuberculosis* culture-positive sputum specimens, 33 (63%) were positive by PCR. The uncorrected PCR sensitivity was 83.5%, with a specificity of 99.0% and a positive predictive value of 94.2%. See Tables 7 and 8 for details of discordant results.

**Analysis of discrepant results.** We examined three interrelated variables in detail to account for the discrepancy in the culture-positive, PCR-negative specimens: the source of the specimen, the concentration of bacteria in the original specimen, and the presence of inhibitors. As shown in Table 4, proportionally fewer sputum specimens yielded culture-positive, PCR-negative results (15%) than did bronchial wash specimens and specimens from other sources (33% each).

The concentration of bacteria in the original specimen can be estimated by both the density of growth and smear results. In Table 5 we compare growth on original medium with PCR results for specimens positive for *M. tuberculosis* and show that the higher the growth intensity, the greater the likelihood of a positive PCR result. Only 17% of the *M. tuberculosis* culture- and PCR-positive specimens showed <1+ growth, while 68% of the culture-positive, PCR-negative specimens were in this category of growth intensity. In the >1+ growth category, 111 of 115 (96.5%) specimens were PCR positive. All four culture-positive, PCR-negative specimens with this much growth were found to contain inhibitors. In the <1+ growth category, 31 of 56 (55.3%) of the specimens were PCR positive. The observation that the concentration of organisms is of primary importance is extended, in that 28 of 37 (75.6%) of the culture-positive, PCR-negative specimens had negative smears (see Tables 3 and 7) but only 45 of 181 (24.8%) of the culture-positive, PCR-positive were smear negative.

**(i) Inhibition.** We also examined specimens for the presence of inhibitors (Table 6). Of the 31 discrepant culture-positive, PCR-negative specimens available for testing, 4 of 20 (20%) sputum specimens and 1 of 11 (9%) specimens from other sources contained inhibitors (16.5% overall). In the random selection group, 0 of 22 (0%) sputum specimens and 3 of 19 (16%) specimens from other sources contained inhibitors (7.3% overall).

**(ii) Culture-positive, PCR-negative specimens.** Table 7 summarizes the characteristics of the *M. tuberculosis* culture-positive, PCR-negative patients. The patients were divided into three groups, which were determined by the concentration of *M. tuberculosis* cultured from their specimens; the organisms from group 1 patients had >1+ growth, those

TABLE 2. Detection of *M. tuberculosis* from 1,166 clinical specimens by direct microscopy and PCR compared with detection by culture

| Culture result (no. of specimens) | No. of specimens with the following PCR result (% smear positive): | |
|---|---|---|
| | Positive | Negative |
| Positive for *M. tuberculosis* (218) | 181 (74) | 37 (24)[a] |
| Negative for *M. tuberculosis* (948) | 11[b] | 937[c] |
| Total | 192 | 974 |

[a] Details in Table 7.
[b] Details in Table 8.
[c] Of these, 140 grew MOTT.

TABLE 3. Smear and PCR results for cultures positive for *M. tuberculosis*

| Fluorochrome smear result | No. of specimens with the indicated PCR result: | | | |
|---|---|---|---|---|
| | All specimens | | Sputum | |
| | PCR positive | PCR negative | PCR positive | PCR negative |
| Positive | 136 | 9 | 121 | 6 |
| Negative | 45 | 28 | 33 | 19 |

TABLE 4. Distribution of specimens that were culture positive for *M. tuberculosis*

| PCR result | No. of samples from various sources | | | | | |
|---|---|---|---|---|---|---|
| | Sputum | Bronchial wash | Tracheal aspirate | Urine | Other | Total |
| Positive | 154 | 18 | 1 | 3 | 5[a] | 181 |
| Negative | 25 | 9 | 1 | 1 | 1[b] | 37 |

[a] These sources were as follows: pleural fluid, $n = 1$; lung tissue, $n = 2$; arm tissue, $n = 1$; unspecified, $n = 1$.
[b] This was a lymph node aspirate.

from group 2 patients had 1+ growth, and those from group 3 patients had <1+ growth. Inhibition was found in all four specimens in group 1 but in only one specimen in the other groups. For all patients in group 1, the single discrepant specimen was the only negative one among the other specimens that were PCR positive, suggesting that inhibition is not related to a characteristic of the patient but to the individual specimen. On examining the histories of these patients, we could not identify why these particular specimens had inhibitors and why the others from the same patient did not. No patient in group 1 or 2 would have been misdiagnosed by PCR because the other submitted samples (one to three samples) were positive by PCR. Of the eight patients in group 3 who were missed by PCR, the three who had two false-negative PCR results clearly had tuberculosis and at least two positive cultures; all of these were immunosuppressed (two HIV-positive patients and one patient with myeloid metaplasia). Of the five patients with a single false-negative result, the results for two patients were probably because of false-positive cultures; one was a postlobectomy patient with recurrent adenocarcinomas diagnosed at that time and one patient had other cultures negative for AFB but subsequently had a sputum sample that was positive for squamous cell carcinoma (neither patient was treated for tuberculosis). Three patients had tuberculosis that was diagnosed with other cultures or had a positive history for tuberculosis.

The group of false-negative specimens was compared with the other specimens. In all groups, the percentage of nonsputum specimens (mostly bronchial wash specimens) ranged from 22 to 30%, which compares with 16% (37 of 218; Table 4) in the entire *M. tuberculosis* culture-positive group (37 of 218; Table 4). The HIV positivity rate was similar for the patients in the three groups (28 to 33%) and for patients from whom specimens were submitted for AFB culture, but it was higher than that for those in the entire *M. tuberculosis*-positive population at our hospital (18%) (44), reflecting the fact that more specimens are submitted from these patients.

TABLE 5. Comparison of growth intensity using liquid and solid media with PCR results for clinical specimens that were culture positive for *M. tuberculosis*

| Growth intensity[a] | No. (%) of specimens with the indicated PCR result: | | | |
|---|---|---|---|---|
| | All specimens that were PCR: | | Sputum specimens that were PCR: | |
| | Positive ($n = 181$) | Negative ($n = 37$) | Positive ($n = 154$) | Negative ($n = 25$) |
| >1+ | 111 (61) | 4 (11) | 102 (66) | 2 (8)[b] |
| 1+ | 39 (22) | 8 (22)[c] | 32 (21) | 5 (20) |
| <1+ | 31 (17) | 25 (68) | 20 (13) | 18 (72) |

[a] See text for growth intensity category definition.
[b] One sputum specimen showed inhibition of the PCR.
[c] One sputum specimen and one lymph node aspirate grew at >2+ intensity and showed inhibition of the PCR.

TABLE 6. Evaluation of clinical specimens for the presence of inhibitors of PCR

| Group (no. of specimens) | Specimen source | No. tested | No. inhibited[a] |
|---|---|---|---|
| Specimens with false-negative PCR (31) | Sputum | 20 | 4 |
| | Bronchial wash | 8 | 0 |
| | Urine | 1 | 0 |
| | Lymph aspirate | 1 | 1 |
| | Tracheal aspirate | 1 | 0 |
| Randomly selected specimens (41) | Sputum | 22 | 0 |
| | Bronchial wash | 11 | 1 |
| | Stool | 2 | 1 |
| | Ascitic fluid | 1 | 1 |
| | Upper extremity | 1 | 0 |
| | Gall bladder | 1 | 0 |
| | Chest wall swab | 1 | 0 |
| | Urine | 2 | 0 |

[a] For specimens with false-negative PCR results, 20% of sputum specimens and 9% of all other specimens were inhibited; for the randomly selected specimens, none of the sputum specimens and 16% of all other specimens were inhibited.

**(iii) Culture-negative, PCR-positive specimens.** Table 8 summarizes the characteristics of the 10 culture-negative, PCR-positive patients (11 specimens). The first four specimens were processed in the same batch. A faint 317-bp band was seen on the initial gels, but on repeat amplification the agarose gel was negative; products of both amplifications hybridized with the probe. None of these three patients had a history of prior therapy for tuberculosis. None of the other cultures of other samples from these three patients was positive for *M. tuberculosis* or MOTT. We conclude that all PCR results in this group were due to amplicon or bacterial DNA contamination introduced at the time of lysing. The two patients in group 2 had current tuberculosis and were receiving antituberculosis therapy. Both agarose and hybridization test results were strongly positive. We concluded that these were true-positive results.

All specimens in group 3 which gave negative agarose results but positive dot blot hybridization results were received from immunocompromised patients. One patient whose urine was culture negative but PCR positive had had tuberculosis 20 years ago and had recently undergone renal transplantation. Two patients were diagnosed with clinical tuberculosis and had been given antituberculosis therapy; one of these patients was HIV seropositive, with this and other cultures being positive for *M. xenopi*, and for the other patient, there were multiple isolations of *M. avium-M. intracellulare*. Another patient, a 70-year-old postpolio paraplegic, had a chronic pneumonia with two isolations of *M. avium-M. intracellulare*. One elderly man died of "failure to thrive," having had right lower lobe pneumonia; only one specimen was submitted for this patient, and no autopsy was performed. It is possible for *M. tuberculosis* to have been undetected in these patients or for MOTT to yield false-positive hybridization test results. To resolve this question, suspensions of isolates from these patients were lysed, amplified, and detected by agarose gel electrophoresis (Fig. 1A) and Southern hybridization (Fig. 1B). The *M. xenopi* isolates gave a slight band at 317 bp, while the *M. avium-M.*

TABLE 7. Detailed analysis of 37 *M. tuberculosis* culture-positive, PCR-negative specimens from 29 patients[a]

| Group (quantity) | Patient[b] | Specimen | Inhibitor present | Smear result | No. PCR positive/ total tested | HIV positive |
|---|---|---|---|---|---|---|
| One (>1+) | 1 | Lymph | Yes | + | 1/2 | Yes |
| | 2 | Sputum | Yes | + | 2/3 | No |
| | 3 | Sputum | Yes | + | 3/4 | No |
| | 4 | Sputum | Yes | + | 3/4 | No |
| Two (1+) | 5 | Sputum | NT | + | 2/3 | No |
| | 6 | Sputum | No | − | 1/4 | No |
| | 6 | Sputum | No | − | 1/4 | No |
| | 6 | Sputum | No | − | 1/4 | No |
| | 7 | Sputum | NT | − | 1/2 | Yes |
| | 8 | BW | No | + | 5/7 | No |
| | 9 | BW | No | − | 1/2 | NK |
| | 10 | Sputum | NT | + | 5/6 | No |
| Three (<1+) | 11 | Sputum | Yes | − | 2/3 | No |
| | 12 | BW | No | − | 2/3 | Yes |
| | 8 | Sputum | No | − | 5/7 | No |
| | 13 | Sputum | No | − | 1/3 | No |
| | 13 | Sputum | NT | − | 1/3 | No |
| | 14 | Sputum | NT | − | 1/2 | No |
| | 15 | BW | NT | + | 0/1 | No |
| | 16 | Sputum | No | − | 0/2 | Yes |
| | 16 | BW | No | − | 0/2 | Yes |
| | 17 | Sputum | No | + | 2/3 | No |
| | 18 | Tracheal aspirate | No | − | 1/2 | No |
| | 19 | Sputum | No | − | 0/2 | No |
| | 19 | Sputum | No | − | 0/2 | No |
| | 20 | Sputum | No | − | 4/5 | Yes |
| | 21 | Sputum | No | − | 0/1 | No |
| | 22 | Sputum | No | − | 0/1 | NK |
| | 23 | BW | No | − | 2/3 | Yes |
| | 24 | Sputum | No | − | 3/4 | Yes |
| | 25 | Sputum | No | − | 2/4 | NK |
| | 25 | Sputum | No | − | 2/4 | NK |
| | 26 | BW | No | − | 0/2 | Yes |
| | 26 | Sputum | No | − | 0/2 | Yes |
| | 27 | BW | No | − | 0/1 | NK |
| | 28 | BW | No | − | 0/1 | NK |
| | 29 | Urine | No | − | 2/3 | NK |

[a] Abbreviations: BW, bronchial wash; NT, not tested; NK, not known.
[b] Several patients were misdiagnosed by PCR, as follows: for patients 15, 21, 22, 27, and 28, the single specimen that was examined was missed; for both specimens from patients 19 and 26, both specimens examined were missed.

*intracellulare* isolates showed a band at about 180 bp. However, neither species gave positive hybridization results by the Southern blotting technique (Fig. 1B).

## DISCUSSION

Although there have been numerous studies of the sensitivity and specificity of PCR for detecting *M. tuberculosis* in cultures or limited numbers of clinical specimens, there have been few studies on its usefulness for large numbers of routine clinical specimens with a high proportion of negative specimens which might yield a higher number of false-positive results. The present study was therefore designed to assess PCR in a large-volume clinical microbiology laboratory setting, allowing for normal work flow and turnaround times (7).

We compared PCR on selected and unselected clinical specimens with sensitive stain and culture methods and investigated the clinical significance of discrepant results. However, in the evaluation of the PCR results, one should carefully analyze the stain and culture standards against which the test will be measured. Our rate of smear positivity of 71% for sputum specimens that grew *M. tuberculosis* was higher than that reported by others (19, 25), probably because of differences in technology (the fluorochrome stain after digestion and centrifugation is more sensitive than the Ziehl-Neelsen stain), patient populations (adults have greater numbers of bacteria than children), and care in reading test results (we read the results at ×500 for 10 min). However, this rate is similar to that reported by Lipsky et al. (27) and Savic et al. (34), who used less sensitive culture methods. The smear results also vary with the species of mycobacteria, as we have shown here.

There can also be variation in the efficiency of detection of *M. tuberculosis* by growth, although this method is usually used as the standard in evaluating PCR. For example, since we use growth in both BACTEC bottles and solid media and our digestion method is less harsh than some, our growth method may be more sensitive; all of our smear-positive specimens (except two from treated patients) grew mycobacteria on culture.

TABLE 8. Analysis of 11 PCR-positive, culture-negative specimens from 10 separate patients

| Group | Patient no. | Clinical tuberculosis? | Prior tuberculosis? | HIV positive? | No. of concurrent cultures tuberculosis positive/total no. tested | Specimen | Intensity | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Agarose | Hybridization | |
| DNA contamination | 1 | No | No | No | 0/7 | Sputum | Faint | Strong | All specimens in this group were processed on the same day |
| | 2 | No | No | No | 0/3 | Sputum | | | |
| | 2 | No | No | No | 0/3 | Sputum | | | |
| | 3 | No | No | No | 0/1 | Bronchial wash | | | |
| True positive | 4 | Yes | Yes | No | 0/3 | Sputum | Strong | Strong | On tuberculosis medication; previously PCR positive |
| | 5 | Yes | Yes | Yes | 3/4 | ? | Strong | NT[a] | On tuberculosis medication |
| Questionable | 6 | No | Yes | No | 0/3 | Urine | Faint | Strong | Renal transplant; on cylosporine |
| | 7 | Yes | No | Yes | 0/2 | Sputum | Faint | Strong | *M. xenopi* isolated three times |
| | 8 | Yes | ? | ? | 0/1 | Sputum | Faint | Faint | 80 yr old; right lower lobe pneumonia; died; no autopsy |
| | 9 | No | No | No | 0/1 | Sputum | Negative | Faint | 70 yr old; *M. avium-M. intracellulare* was isolated two times |
| | 10 | Yes | No | No | 0/3 | Sputum | Negative | Faint | *M. avium-M. intracellulare* was isolated three times; on tuberculosis medications |

[a] NT, not tested.

The 37 false-negative results (culture-positive specimens not detected by PCR) were traced to low numbers of organisms in the specimen or the presence of inhibitor. We examined whether the lack of amplification was a property of the organism itself but subsequently found that all of these strains could be amplified from culture. However, the different degrees of sensitivity (some of the specimens showing sparse growth were detected by PCR, while others were not) might be related to the number of copies of IS*6110* in the strain; the number of copies of this insertion sequence can vary from 10 to 16 (6, 13).

Other studies with the same IS*6110* target but sometimes with a smaller amplified sequence have reported greater sensitivity (3, 14). However, to achieve this, special procedures had to be initiated. Brisson-Noel et al. (13) had to perform phenol extraction and, in some cases, lysis in guanidium thiocyanate. In some reports, the percentage of known positive samples was artificially high (12, 14). In others, the culture was questionable (sending specimens from Sri Lanka to The Netherlands might lead to decreased viability but not decreased DNA content [26]). In our study and in other studies (31, 38, 41), there was a discrepancy between the sensitivity of detection from cultures and from patient specimens. The problem may be in achieving complete lysis of the tubercle bacilli from most clinical samples. Both sonication (5) and specimen heating (8, 45) have been reported to be suitable methods for achieving lysis. The lack of sensitivity does not seem to be a problem for CSF specimens, for which there are more positive PCR results than culture results (10, 35, 36, 45); we also observed a positive PCR result after removal of inhibitor for a patient who had a negative culture done elsewhere (data not included because they were from a non-Veterans Affairs patient).

A second factor in false-negative reactions is the presence of inhibitor. In the present study, undefined inhibitors of amplification reactions were detected in 0 to 20% of the clinical specimens, depending on the source and the selec-

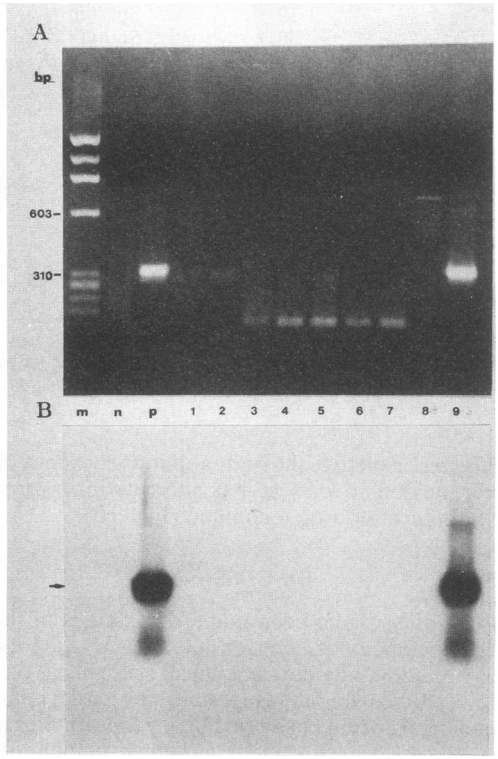


FIG. 1. Agarose gel electrophoresis (A) and Southern hybridization with alkaline phosphatase-labelled probe (B) using amplified products from mycobacteria cultured from patients in whom original specimens were associated with false-positive PCR results (Table 8). Lane m, *Hae*III-digested ϕX174 used as size marker; lane n, template-negative control; lane p, template-positive control; lanes 1 and 2, *M. xenopi*; lanes 3 to 8, *M. avium* complex; lane 9, *M. tuberculosis* clinical strain isolated from a patient known to have tuberculosis. The arrow in panel B indicates the expected position of the 317-bp amplified product.

tion. The overall rate was comparable to the rates of 7.9% reported by Soini et al. (39) and 5% reported by Brisson-Noel et al. (4). We did not attempt to identify the nature of inhibitors, but it has been suggested that substances such as heparin, hemoglobin, phenol, and sodium dodecyl sulfate may be potent inhibitors (17, 22, 29). Unlike the report by Brisson-Noel (3), who found the inhibition to be patient related in some circumstances, we noted that the inhibition phenomenon was not patient related, because other specimens from the same patients were amplifiable.

In the present study (Table 8), most false-positive specimens could be attributed to DNA contamination (patients 1 to 3) and the presence of nonviable organisms in patients treated for tuberculosis (patients 4 and 5) or patients with reactivated tuberculosis (patients 6 and 9). Since the other three specimens (weakly positive by hybridization) all grew MOTT, there may have been false-positive hybridization results, although reamplification of the cultures did not yield positive hybridization results. In agarose gels, the bands can be detected for both *M. xenopi* (at 317 bp) and *M. avium-M. intracellulare* (approximately 170 bp). The overall rate of MOTT isolation (not counting *M. gordonae*) was 6%, but four of the five unexplained false-positive samples grew MOTT. Similar to the findings of Githui et al. (18) that HIV positivity in patients with pulmonary tuberculosis is not associated with significant differences in smear positivity rate, culture positivity rate, or drug resistance, we did not find a difference in the PCR positivity rate related to HIV status. We did, however, find the rate of HIV seropositivity to be slightly higher in the false-positive group.

If corrections are made for technical error (amplicon or genomic DNA contamination), presence of inhibitor, false-positive cultures, and culture-negative patients with findings compatible with disease, the sensitivity, specificity, and positive predictive value of our PCR are 86.1, 99.7, and 98.4%, respectively. If, in addition, the results for all specimens examined from a single patient are considered in total (as cumulative), the sensitivity, specificity, and positive predictive value become 95, 100, and 100%, respectively. This compares to a sensitivity and positive predictive value for *M. tuberculosis* of 66 and 72%, respectively, for the fluorochrome-stained smear alone. In addition, 71% of the smear-positive specimens were *M. tuberculosis*.

Rapid results with PCR could have a large impact on hospital costs. From Table 1, we see that if all the patients who have a AFB-positive sputum specimen are placed under AFB isolation precaution, 28% of these will be unnecessary because they do not have *M. tuberculosis* and 29% of people who have tuberculosis will not be in isolation because they had a negative AFB smear. On the other hand, if PCR were performed on the same day, none of the people with specimens that grew MOTT would have been put in isolation, and only 19 of 179, or 11%, of the actual tuberculosis patients would not be in isolation. As we have noted, these patients (except for those with inhibitors in their specimens) tend to have fewer organisms in their sputa and may be less infective. In this discussion, we have concentrated on sputum specimens because they are the specimens most commonly obtained and have the most public health significance.

In defining the limitations of PCR for large-scale clinical use, we found that formation of the 317-bp product is 100% specific, but there is a minor band which can occur with *M. xenopi*. This could be a problem if the expected incidence of *M. xenopi* isolation were high and no culture or hybridization were done. In addition, if a dot blot hybridization is performed directly on the amplification products, low-level hybridization with *M. avium-M. intracellulare* products may occur. An undefined inhibitor can be present in an average of 7.5% of specimens, but this varies with the source of the clinical specimens. Even though the PCR described here is not as sensitive as culture, if three or more specimens are processed, essentially no patients with tuberculosis would go undetected and no patients with AFB-positive sputum would be undiagnosed. This bodes well for the use of PCR for the quick identification of the most infective people so that proper isolation and therapy can begin.

## ACKNOWLEDGMENTS

The contributions of Robin Heubner, the Centers for Disease Control and Prevention, and Gregory Buffone, Nelda Wray, and Chris LaHart of Baylor College of Medicine are gratefully acknowledged.

The study was supported by CDC grant U52/CCU606005-1.

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